3

A Study of the Structure and Development of Certain Reproductive Tissues of *Mugil cephalus* Linnaeus[1]

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(Plates I-VIII)

INTRODUCTION

ONE of the more common species of the South Atlantic and the Gulf of Mexico is *Mugil cephalus* Linnaeus, the black mullet, gray mullet or jumping mullet. It is also an important food fish of the warmer waters of China, Japan, India, Australia, South Africa, the Hawaiian Islands and the Mediterranean Sea. Because of its importance as a source of animal protein and its adaptability to pond culture, an increasing interest is being given to the propagation of this mullet abroad. Considerable investigation is being undertaken by government-sponsored projects on the mullet fishery, particularly in the Far East. In the United States, especially along the Gulf of Mexico coast where the mullet fisheries are of economic importance, a number of studies of the species have been published by state and national fishery bureaus. The State of Florida Board of Conservation and Miami University especially have been interested in various aspects of the Florida mullet fisheries.

The natural history of *Mugil cephalus* and fishery data are treated quite extensively in the following: Jacot (1920), Smith (1935), Kilby (1949), Kesteven (1942, 1953), Broadhead (1953), Thomson (1949, 1951, 1953), Gunter (1945), Roughley (1951), and Sarojini (1951). Little detailed, authentic information exists on the spawning habits of *Mugil cephalus*, however, and practically nothing is recorded on its embryology and organogenesis. Mullet eggs of various species have been described by Cunningham (1891-1892), Errenbaum (1909) and Sanzo (1936). The latter describes *M. cephalus* eggs (size, color, oil drop, etc.), fertilization, cleavage, hatching and the gross appearance of the young fry to the eighth day. The recent observations of Nair (1957) are similiar in scope and generally agree with Sanzo's 1936 report. No histological studies were made by either investigator. Dekhnik (1953) also describes the eggs of *M. cephalus* and three other species of Black Sea mullets. Jacot (1920) reports on the development of the young mullet in his study concerned with migration, scale annulation and the development of external features.[2]

Mugil cephalus appears to have no external sex markings or structures; sexing of the fish, except in the spawning season, is done by gross examination of the gonads. Broadhead (1953) has adapted the Australian scheme of sexing (Kesteven, 1942) to the Atlantic and Gulf of Mexico mullets. In the course of extensive experimentation on the pond cultivation of *M. cephalus* in brackish water at Marineland, Florida, Johnson (1954) found evidence to indicate that the sexing of this mullet, particularly the immature fish, was probably subject to some error. He also found some evidence– the presence of oocyte-like cells in young fish–that suggested

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[2]Concerned with a closely related species of the Atlantic (*M. curema*) is the report of William W. Anderson (1957) on the "Early Development, Spawning, Growth, and Occurrence of the Silver Mullet (*Mugil curema*) along the South Atlantic Coast of the United States," U. S. Dept. Interior, Fish and Wildlife Service, Fish. Bull. No. 119, vol. 57: pp. 397-414. The author also reports in the same volume (Bull. 120, pp. 415-425) on "Larval Forms of the Fresh-water Mullet (*Agonostomus monticola*) from the open ocean off the Bahamas and South Atlantic Coast of the United States."

that the mullets might be hermaphroditic, possibly protandrous or protogynous. The literature discloses practially no information on the anatomy and histology of the reproductive system of the Mugilidae. Broadhead (1953) and others have commented on this lacuna. This study was undertaken to describe the anatomy and histology of the gonads of *Mugil cephalus*. In order to ascertain if some form of hermaphroditism existed in this mullet, a development study of the gonads from the earliest stage obtainable (20 mm. S. L. fry) to the mature fish was undertaken.

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Materials and Methods

Tissues for histological examination were secured from fish cast-netted from the ocean and the Metanzas inland waterway, adjacent to the laboratory at Marineland, Florida.

Fish used in this study ranged in size from 19 mm. to 425 mm. Measurements of the large specimens were made on a standard board, and standard lengths were determined. Specimens under 100 mm. in length were measured after fixation and partial dehydration in 80% ethanol. Small fish were measured by means of a dissecting microscope. The gonads and adhering mesogonium were removed from the large fish immediately after death, washed for a few seconds in sea water to rid them of sand and grit, and stretched on strips of index card. After identification, they were placed in fixing solution. Small fish were anesthetized in 0.2% solution of Tricain methanesulfonate (MS-222) in sea water; the abdomen was then incised and the fish placed in the fixing solution.

Bouin's solution was used for the bulk of the material. Some tissues were fixed in 10% formalin in sea water; formol-fixed material was generally unsatisfactory since it exhibited relatively great shrinkage and poor staining quality.

After 24-hour fixation, the tissues were transferred to 70% ethanol and sent to New York. Here they were placed in 80% ethanol for at least 24 hours. By means of a dissecting microscope the small fish were measured and the fins and skin removed to facilitate sectioning. It was possible to remove the peritoneum with the adhering gonad from the 30 to 90 mm. specimens.

Dehydration was accomplished by the Zirkle Normal Butyl Alcohol series (Krajian, 1940, p. 212). Infiltration (two baths) and final embedding in fresh paraffin was in Fischer (56°-58°) Tissuemat. Routinely, sections were cut at 7 and 5 micra.

Two staining methods were utilized as routine procedure. The Alum Hematoxylin of Galigher (a modification of the Harris Hemalum) was used in conjunction with Triosin (0.5% in 90% ethanol), buffered with N/10 HCl (approx. pH 5.4), as recommended by Galigher (1934), gave nice differentiation of nuclei and cytoplasm. Some sections were stained with Lillie's Mayer Alum Hematoxylin, (Lillie, 1954, p. 76) and counterstained with Triosin. Some slides, in many instances alternate slides in a series, were stained with Masson's Ponceau-acid fuchsin, Anilin blue or light green technique (Lillie, 1954, p. 351). However, 1% phosphotungstic acid was substituted for phosphomolybdic acid. The Gomori elastin stain (Lillie, 1954, p. 364) was used to identify elastic connective tissue. The Krajian (1940, p. 111) version of the Foot reticulum stain proved satisfactory for demonstrating reticulum.

All stained sections were mounted in a solution of Clarite in Xylene.

Description

Morphology and Histology of the Indifferent Stages

20 mm. Fry, Collected Nov. 28-Dec. 24.—The gonad primordium of the 20 mm. fry consists of two microscopic fibers suspended from the pigmented peritoneum on either side of the dorsal mesentery. The strands form a "V" with its apex slightly anterior to the cloaca; each arm extends anteriorly the length of the abdominal cavity, *i.e.*, approximately 4 mm. The peritoneum with the attached genital primordium separates the abdominal cavity from the dorsal body cavity in which the swim bladder and mesonephros are developing (Figs. 1 and 1a). Transverse sections through the anterior abdominal region reveal the progonal masses as club- or leaf-shaped aggregates of mesenchymal cells enclosed in a reticulum-like capsule which is suspended from the peritoneum by a slender stalk or stem. The

non-germinal progonal areas contain developing nerves, blood vessels and varying amounts of black pigment. Sections through the converging posterior fibers show them to be essentially similar in shape. The capsule, however, is better developed, particularly the lateral and ventral borders, which are composed of squamous or low columar cells. This epithelium appears to be a proliferation from the peritoneum, via the stalk. At the extreme caudal end, the primordia appear as lateral outgrowths from the dorsal mesentery.

Germinal elements in the posterior primordia are represented by two structures: a cord of tissue which courses through the caudal third of each fiber, and germ cells which are embedded within the cords. In section, the cord is an irregularly shaped syncytium of finely granular, acidophilic material. Nuclei of the cord are round or ovoid and measure no more than 5 micra in diameter.

Germ cells (Fig. 2) lying in the ground cytoplasm of the syncytium are ovoid, relatively clear cells that measure 9 to 12 micra on their long diameter. Germ cell cytoplasm is faintly stippled with acidophilic material, and no basophilic or yolk granules are present. The nuclei are somewhat flattened spheres with their greatest diameter from 7 to 9 micra. The prominent nuclear membrane appears to be dotted with chromatin particles on its inner surface, and fine threads of chromatin anchored to the nuclear membrane form a network in the colorless karyoplasm. A conspicuously large, clear, faintly basophilic, eccentrically placed nucleolus is always present, and usually two or three smaller nucleoli are to be found in the chromatin mesh. An idiosome is usually discernable.

In two specimens in which counts could be made, it was found that one primordium contained 12 germ cells and the other 15. No germ cells were found outside of the gonadal anlage.

35-50 mm. Stage—Mullet fry collected in the latter part of February, in March and in the forepart of April measure from 35 to 50 mm. Sections of the gonad anlagen of these stages show the glands to be of similiar shape to the primordium of the younger fry, but considerably larger. Much of the increase in size is the result of the development of the non-germinal region which occupies the medial side. Sections from the anterior strands show vascular elements occupying most of the non-germinal region. The extreme caudal end of the strands appear as knobs on either side of the dorsal mesentery. Fig. 4, from a 50 mm. specimen, shows the knob-like appearance of the posterior region of the gonads.

Sections through the gonadal strands of a 40 mm. mullet, collected March 27, show the germinal portion of the gonad as an acidophilic band of tissue lying beneath the capsule. The band is similiar to the syncytial tissue described in the 20 mm. stage. A section from the mid-region of the gonad may show five or six germ cells imbedded in the syncytial band.

A 50 mm. specimen (Fig. 3) is essentially similiar in appearance. However, more germ cells are present; a 5-micra section contained 8 cells. Mitotic configurations in the germ cells are encountered with more frequency in this stage. The epithelial border is still generally limited to the lateral side and ventral end.

In specimens as small as 25 mm. there is some indication that cells from the lateral epithelial border of the gland are penetrating into the stroma at the point of juncture of the gland and the stalk. In the mid-region of the 40 mm. stage gonad, this proliferation is evidenced by a cord of flat cells. Subsequently the cord forms an epithelium-lined fissure which separates the gland into a lateral germinal region and a mesial mesogonium. Lateral outgrowths from the fissure form duct-like processes which extend to the germ cells on the periphery of the gland.

61 mm. Stage.—A 7-micra section through a gonad cord from a specimen collected on April 23 is shown in Fig. 5. The lighter-staining stroma of the gland appears to be divided by the darker-staining branches of the duct system. Peripheral germ cells have formed nests along the lateral and ventral borders (right and bottom) and some migration of germ cells in the ducts has taken place (lower right border).

75 mm. Stage.—Fig. 6 is from a section, approximately the mid-region, of a developing gonad from a 75 mm. fish, collected in August. The dark structure to the left of the gonad is the peritoneum. Nests of germ cells occupy most of the lateral edge and ventral end. Nests of germ cells are separated by septa of small cells which appear to be proliferations from the capsular epithelium of the gland. Note the hilus, indicated by an arrow, which appears between the body of the gonad and the stalk. At this point, epithelium from the stalk and peritoneum appears to be growing into the gland. From this ingrowth, a cord and subsequently a branched system of ducts form. The main duct more or less divides the primitive gonad into a lateral germinal region and a medial portion which is the future mesogonium. The latter is composed of developing connective tissue fibers. Note the large vascular elements in the mesogonial area (right). In time this region will also support nerves and small ganglia.

The germ nests along the lateral border of the gland are much larger than those seen heretofore. Some of the large nests, when followed through several sections, may contain as many as fifteen cells. Between the large germ cells are small irregularly shaped cells with sparse acidophilic cytoplasm. It appears that these elements are derived from the syncytial cord and probably from the capsular epithelium. Single germ cells and small groups of two or three cells, which apparently have broken away from the peripheral nests and have moved toward the center of the gland, are conspicuous in this stage.

100 mm. Stage.—Fig. 7 is from a larger specimen collected in February. The increase in the stromal tissue of the germinal and mesogonial regions is marked. Note the main duct which will become the vas deferens in the male.

Fig. 8 is from a section of a gonad of a 150 mm. specimen collected in May. The sex of the individual is not indicated by the structure of the gland. There are nests of germ cells and evidence of cord formation. If the nests form cords, maleness is indicated. The dark cell to the right of the figure and just below the center is one of the ova-like cells which are quite common in developing gonads. They appear to be small oocytes with normal-appearing nuclei. They have a large nucleolus and in some cases the chromatin threads can be seen. The cytoplasm, however, is abnormal. Frequently, the ground substance appears to be broken up and stains acidophilic rather than basophilic, as does the cytoplasm of normal oocytes—possibly an indication of degenerative changes.

The germ cells shown in Fig. 8 vary considerably in size and shape but are essentially similar to the much larger germ cells found in the peripheral nests of the young fry. The most prominent structure of germ cells is the nucleus, which occupies most of the cell. The nuclei always contain a large, eccentrically-placed, faintly basophilic, transparent nucleolus. In favorable sections, two or three small nucleoli can be seen and the chromatin threads are visible. The gonial cytoplasm contains varying amounts of faintly acidophilic particulate matter. With high magnifications, the idiosome may be seen.

The germ cells shown in the illustration do not represent all of the cells present in the area. With high magnification, many more single cells can be seen in the stroma and between the epithelial lining of the ducts and the stroma. Frequently, germ cells are flattened or crescentic in shape; however, their outline (cell membrane) is quite even. There appears to be no evidence that they assume amoeboid shape. This is in contrast to the cells of the ducts which are very irregular. At this stage there does not appear to be any indication of the sexual potentialities of germ cells.

Development of the Testes

Mullet testes from the August collection are slightly larger than those collected in early summer. Histologically, August glands might be characterized as representing a period of germ cell mitosis; only rarely is evidence of meiotic activity found. A rough measurement of the greatest diameter of a testis from a 225 mm. specimen collected in early August is less than 2 mm. The testis from a mullet of the same size, collected on October 28, measured 13 mm. in diameter. Histological examination shows that the October specimen is well on the way to ripeness.

Fig. 9 shows the general appearance of the maturing testes of a 250 mm. mullet collected in late summer. The stroma (the darker areas) consists of connective tissue. Selective staining shows the stroma is chiefly reticular (argyrophilic), collagenous and elastic fibers. A small, somewhat diffuse area of lymphoid-like tissue, appearing in six to eight different 7-micra sections, is usually encountered in the testes. There does not seem to be any uniformity in the size or position of lymphoid areas; they may be near the vas deferens or in the peripheral region. In some instances they appear to be separated from the surrounding tissue by a thin thread-like capsule; in other cases, the boundaries are diffuse.

The mesorchium of the mullet is made up of coarse collagenous fibers. It suspends the testes, supports the vas deferens and contains blood vessels, nerves and ganglia. In its caudal extremity the mesorchium becomes a heavy sheath that encloses the sperm duct which has formed by the fusion of the two vas deferentia. This anastomosis occurs a few millimeters from the cloaca.

The seminiferous tubules or sperm ducts, which are actually main and secondary branches of the vas deferens into which germ cells have lodged, project into the stroma. Fig. 10 shows the distal portions of the tubules from the August collection. The duct epithelial lining has been so disarranged by the plethora of germ cells as to have lost its morphological identity. The lumen of the tubules, frequently almost occluded by germ cells, becomes continuous with alveoli which develop in the peripheral germ cell nests.

The maturation of mullet male germ cells would appear to be similar to the pattern described in the perch by Turner (1919), and in *Cottus* by Hann (1927). After a period of cell

multiplication (August and September) the germ cells appear to shrink in size. This decrease seems to be caused by a diminution of the cytoplasm. The cytoplasm also stains with less intensity, although it is still acidophilic. Each small cell, presumably a spermatogonium, divides a number of times and forms an aggregate or cyst. Turner (1919) suggests that as many as six divisions occur in order to give rise to the number of spermatocytes in a cyst. This would seem to be true of the mullet. Turner (1919) and Hann (1927) report that each cyst has a fine membranous capsule. Hann finds the capsule somewhat difficult to resolve. This difficulty is also encountered in the mullet; a well-delineated and complete capsule does not seem to exist. From the incomplete capsules examined, the impression is formed that the capsule is composed of flattened duct epithelial cells. Fig. 11 shows a number of cysts in the tubules from the October collection. In some testes, degenerating cysts are numerous; they are compact aggregates of pycnotic nuclei in an acidophilic matrix. Turner (1919) finds degenerating spermatogonia in the perch.

The small size of the nuclei, and possibly unsatisfactory preservation, precludes a detailed description of maturation stages. Synaptene nuclei are evident but subsequent stages are impossible to identify. Young spermatids appear to be ovoid cells, less than 2 micra in length, with crescentic nuclei. Clusters of newly developed sperm appear in section as fan-shaped, or as Turner (1919) describes them, "parachutes". Fig. 13 contains several examples. It would seem that sperm in the "parachute" stage are not enclosed within a cyst; at least no membrane can be detected. Presumably the formation is maintained by the adhesion of the sperm tails. In ripe testes, spermatoza occur as dense, unorganized masses (Figs. 12 & 13) in the tubules and the vas deferens.

The Spent Testes

Fig. 14 from a 215 mm. fish is of an area from a spent testis. The general disarrangement of the tubules has resulted from a release of sperm and shrinkage of the organ. Under higher magnification one can identify small but typical germ cells in the walls of the tubule epithelium (circled area, Fig. 14). Nests of germ cells are on the periphery; many of the cells appear to be dividing. Fig. 15 shows a low power view of a section of spent testis from a larger specimen (275 mm.) The crenated edge of the gland is characteristic, The tubules are separated by coarse strands of connective tissue. There are fewer germ cells in the tubule epithelium of this specimen and the nests appear to be less in number and generally smaller. *i. e.*, they have fewer cells than the nests in Fig. 14. The paucity of germ cells suggests senescence.

In Fig. 14 the dark cells are two oocyte-like cells. Note that they have broken out of the nests. In Fig. 16, from the same specimen, is an oocyte-like cell in the vas deferens; note the sperm. This cell has the appearance of an immature, normal oocyte.

Development of the Ovary

The development of definitive ovaries in *Mugil cephalus* is indicated in fish measuring from 175 to 225 mm. standard length.

The gross macroscopic appearance of a developing ovary is similar to the immature testes, *i. e .*, two triangular-shaped strands of tissue suspended from the peritoneum anteriorly and the dorsal mesentery posteriorly (Fig. 17). With the increment and growth of oocytes the potential lobes of the ovary become cylindrical in shape and are enclosed in smooth muscle tunicae; positive identification is then possible.

Before gross changes in the presumptive ovary are discernable, it may be identified microscopically by the arrangement of the germ cells. Germ cells originating in the peripheral nests, which have migrated into the lumina of the ducts, undergo a number of divisions as undifferentiated germ cells, as in the developing testis. Ovarian differentiation is evidenced by the incorporation of a number of germ cells into a nest or nidus (Fig. 19). A nest may contain more than twenty cells of varying degrees of maturity. It is not unusual to find germ cells, oogonia and oocytes in the same nest. The transformation of germ cells into oocytes, like the formation of spermatocytes, is preceded by a diminution in the size of the germ cells followed by several mitotic divisions which form small oogonia. Young oocytes are made conspicuous by the basophilic ring of yolk material which forms around the nuclei.

A nest of germ cells, gonia, etc., is enclosed in a fine membranous capsule. The source of this capsule appears to be the duct epithelium and probably stromal cells. Within the nests are small, flat cells—similiar to duct epithelial cells —which become oriented around the oocytes. It is from these cells that the follicle cells develop.

Fig. 18 is from a caudal section of a 200 mm. length mullet, collected in February. The two lobes of the ovary are attached to the dorsal mesentery in this region. The mesovarium is much less developed than the mesorchium. Much of the stroma of the gland has been supplanted by the developing oocytes. Nests of oocytes have

broken down and finger-like lamellae of oocytes are forming. The fissure between the mesovarium and the glandular region (the vas deferens in the male) is becoming occluded by the increased volume of the ovary proper. Fig. 19 shows a small area from an anterior section of the same gland. The field is made up of maturing oocytes and some mature cells. The stroma of the ovary has been practically obliterated by the germ cells.

Fig. 20 shows the lamellae of a more mature ovary. The lamellae appear as hollow finger-like projections.

The development of the muscular tunica externa or capsule of the ovary proceeds in two stages. The thin serosa of the undifferentiated gland is overgrown by a pigmented connective tissue capsule which appears to originate in the stalk or presumptive mesovarium. Fig. 18 shows this overgrowth.

In the stage of development represented in Fig. 18, the tunica has an inner lining of squamous cells, an inner and an outer layer of collagenous and elastic connective tissue which run more or less longitudinally. Between the connective tissue layers are blood vessels, nerves and small ganglia. Islets of black pigment cells are enclosed in the connective tissue. The serosa of the gland is a pigmented layer which in places is several cells deep.

As development of the ovary proceeds, muscle fibers appear in the connective tissue capsule. They appear first near the mesovarium. In the near-ripe and ripe ovary the tunica is composed of two or three layers of smooth muscle which appear in transverse sections of the ovary to be running obliquely (Fig. 20). The ripe ovary does not appear to be pigmented and sections of the tunica do not show the pigmented serosa of the earlier stage. If a small piece of the tunica is examined with a dissecting microscope, the serosa is seen to contain regions of dispersed pigment cells.

The Ripe Ovary

Oocytes from ripening ovaries, collected in the early fall, show the coalescence of the fine, basophilic, cytoplasmic granular material into larger yolk granules. In the mature oocyte, a thin layer of the fine yolk precursor is retained beneath the cortex: this is shown in Fig. 23 as a dark layer beneath the zona radiata. Note also in Figs. 22 and 23 the clear round areas in the cytoplasm; presumably, they represent the spaces left by the dissolution of the oil. Sanzo (1936) reports that the eggs of *M. cephalus* have a diameter of 0.72 mm. and contain a polar oil drop 0.28 mm. in diameter.

The germinal vesicle occupies a central position in the oocyte; its membrane is wrinkled and uneven in contour. Nucleoli are arranged around the periphery of the vesicle—as many as fifteen may be seen in median, 10-micra sections. Lampbrush chromosomes are discernible in the finely stippled karyoplasm. Fig. 22 shows the peripheral regions of three oocytes. The conspicuous broad zona radiata has a typical radiate appearance. No vitelline membrane is detectable in the mullet oocyte. In sections stained with Gomori's elastin stain, the zona radiata appears to have a thin peripheral region which is stained by the fuchsin-aldehyde reagent.

The follicular epithelium of the mature oocyte is a membranous-like capsule of flat squamous cells (Fig. 22). Sections stained with reticulum stain show fine fibers surrounding the follicle.

The Spent Ovary

Fig. 23 is from a partially spent ovary collected in February from a fish of 225 mm. standard length. The lamellae are partially collapsed. The follicle cells of spent ovaries appear as strands and clumps of shrunken cells. The finer ovarian blood vessels also appear to be degenerating. Fig. 23 contains many atretic follicles; their ultimate course is not clear; presumably they are resorbed. Note also the small, dark young oocytes close to the lamellar membranes. Fig. 24, from a 275 mm. female, shows a small area from a spent ovarian lamella.

A Testis-Ovary

A single instance of hermaphroditism was found in the October 8 collection. The specimen was 250 mm. in length and by gross appearance of the gonads was sexed as a female. A section of one ovary is shown in Fig. 25. The other ovary contained fewer areas of male elements; in fact, many sections appear to be those from a normal ovary. At the top and to the right in Fig. 25 is a slit-like lumen. This appears to be a collapsed vas deferens; in normal ovaries this is not present. Most of the oocytes appear to be normal; however, small degenerating oocytes are numerous. In the figure the male region is most pronounced in the gray area to the left of center. This testicular area appears to be disorganized; if a tubular arrangement was present, it has been disrupted. Fig. 26 shows a small area from the male region. Note the normal appearance of the sperm parachutes and the several small oocytes. The latter appear abnormal although healthy-appearing oocytes can be found in close proximity to male elements. The suspensory tissue closely resembles a mesovarium and the muscular tunica is typically ovarian.

Discussion

Origin of Germ Cells

The literature on the ontogeny of vertebrate germ tissue reveals that during the past three-quarters of a century three general theories have been advanced to account for the origin of ova and sperm. The initial and oldest theory, that of the "germinal epithelium," which was originally proposed by Waldeyer in 1870 and according to Witschi (1948) abandoned by Waldeyer in 1906, proposed a sematic source of germinal elements. Nussbaum is generally credited with a second theory which proposed that germ cells are extra-embryonic in origin. Finally the idea has developed that extra-embryonic cells give rise to germ cells, but that they are supplanted or supplemented by germinal epithelium or other cells of somatic derivation. The often-quoted review by Heyes (1931) summarizes the literature pertaining to the origin of the vertebrate germ tissues to 1931. Everett (1945), Gillman (1948), Witschi (1948), Nieuwkoop (1949), Johnston (1951) and Nelson (1953) have reviewed the subject in the light of recent investigations.

Origin of Germ Cells in Fishes

Part of the literature dealing with the theories on the origin of germ cells and tissues in the vertebrates is concerned with the condition in fishes and indicates a similar division of opinion on the question of the genesis of germinal elements.

Followers of the original germinal epithelium theory of Waldeyer are in the minority; they include Hoffman (1886) and Bohi (1904). The second school of thought, which proposes a strict adherence to the Nussbaum theory, *i.e.*, a single source of germ cells, the primordial germ cells being of extra-embryonic origin, represents the majority of investigators, including Eigenmann (1891), Beard (1900), Allen (1911), Bachman (1914), Dodds (1910), Okelberg (1921), Hann (1927), Stromstein (1931), Johnston (1951) and Robertson (1953). Finally, there are those who admit the existence of primary germ cells, but who believe that sperm and ova arise from somatic cells as well. To this group of investigators belong Essenberg (1923), Foley (1927), Butcher (1929), Wolf (1931), Odum (1936) and Guerbilsky (1939).

Study of the development of the mullet gonad, from its appearance in the 20 mm. fry through maturity, indicates that the germ cells found in the 20 mm. stage are the antecedents of ova and sperm. As to the source of these primary germ cells, eggs or embryos not being available, one cannot say whether they are extra-embryonic, from an early cleavage stage as Eigenmann (1891) reported in *Micrometrus aggregatus*, or from the more common source, the gut-yolk sac endoderm; or whether they did not develop from peritoneal epithelium, as described by Hoffman (1886) in the salmon. If the large germ cells in the 20 mm. mullet fry are somatic, it might reasonably be expected that even in such a comparatively late stage there would be transitional stages between capsule or stromal cells and germ cells. Such was not the case.

Investigators generally have made a point of the fact that primary germ cells have no single morphological characteristic that makes them unique and identifiable.[3] They are usually referred to as large cells, sometimes as the largest cells in the embryo. Johnston (1951) gives a table of primordial germ cell dimensions, as reported in various forms. Comparison of Johnston's data and the reports of others indicates that the mullet germ cells, measuring 9 to 12 micra in diameter, are quite typical of the size of primary germ cells.

No yolk granules were seen in the germ cells of the mullet fry. The presence of yolk in the primary germ cells of vertebrates has been emphasized as a distinguishing characteristic by some authors, *e.g.*, Beard (1900) and Burger (1937). Generally, investigations on fish development bear this out. In some instances the germ cells retain some yolk after dividing in the gonocoel. On the other hand, Dodds (1910) found no yolk in the primary germ cells of *Lophius*, and Johnston (1951) reports the same condition in the freshwater bass. Both investigators traced the germ cells back to a premigration period.

Johnston (1951) states that he was able to differentiate primary germ cells from blood cells which they are said to resemble. Jordan (1917) and Risley (1933) stress the similarity of germ cells and blood cells. In the mullet, the extreme dissimilarity in size of the two types of cells gave no cause for confusion.

Germ Cell Nuclei and Nucleoli

Lobed nuclei are not characteristic of the mullet germ cells, as reported in *Raja* by Beard (1902), in the toadfish by Sink (1912) and in the guppy by Goodrich, Dee, Flynn & Mercer (1934).

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[3]McKay, Hertzig, Adams & Danziger (1953) report that human primordial germ cells are positive to the alkaline phosphatase reaction. Subsequently, Chiquoine & Rothenburg (1957) have found that the primordial germ cells of the chick and *Ambystoma* are negative to this test. These reports suggest the possibility of phylogenetic differences in the phosphatase characteristics and further investigation of this subject seems warranted.

Most descriptions of germ cells note the presence of two or three nucleoli, as in the mullet. Dodds (1910) describes the extrusion of nucleoli in the early germ cells. He suggests that this phenomenon is a unique characteristic of germ cells. No evidence of nucleolar extrusion was seen in the germ cells of *M. cephalus.*

The Genital Strand

The position and general morphology of the genital strands in the young mullet are essentially the same as has been described in other fishes. However, the strands in mullet fry appear to have developed independently of the presence of germ cells. These strands are reported to be interrupted in the lamprey by Okelberg (1921) and in the bass by Johnston (1951). The latter compares the early gonad of the bass to a string of beads. Each bead might be said to represent a local proliferation of the peritoneal epithelium which has been evoked by the primordial germ cells. In the mullet, as in Fundulus (Bachman, 1914), in the toadfish (Odum, 1936), and in some other forms, the genital strand is not confined to areas which contain germ cells. From this fact and from the presence of a progonal region which is the supporting structure for nerves and blood vessels, one might infer that the strand did not develop in response to the presence of germ cells. Burns (1955) cites several studies, principally based on amphibian experimentation, which indicate that germ cells alone cannot induce the genital ridge and are not essential for its origin.

Maturescence of the Testes

The inception of maturescence in the gonads of *Mugil cephalus* is essentially the proliferation of germ cells from the peripheral nests toward the center of the gland. In the developing testes this proliferation contrives to form the tubules of germ cells within the walls of the duct system. This is the pattern reported for a number of forms, *e.g.,* Turner (1919) in the perch; Hann (1927) for *Cottus;* Stromstein (1931) in the goldfish; Goodrich, Dee, Flynn & Mercer (1934) in the guppy; Wolf (1931) in the platyfish; Jones (1940) in the salmon; and Johnston (1951) in the largemouth bass. Descriptions of testes in a number of other fishes indicate a similar architecture — stickleback, Craig-Bennett (1930); *Betta splendens,* Bennington (1936); largemouth bass and bluegill, James (1946a); goldfish, Kinoshita (1933); *Fundulus,* Mathews (1938), salmon, Weisel (1943) and Jones (1940).

Turner (1919) notes that the tubules (he refers to them as lobules) resemble the mammalian seminiferous tubules. Stromstein (1931) refers to them as seminiferous tubules. Bullough (1939) and Craig-Bennett (1930) state that they are not true seminiferous tubules. Craig-Bennett (1930) finds that the tubules of the stickleback contain no permanent germinal epithelium. In the mullet, residual germ cells are present in the post-spawned tubules and in the peripheral nests. Hann (1919) notes a similar condition in *Cottus* and Bennington (1936) in *Betta splendens.*

Turner (1919) and Foley (1927) report that male germ cells have their origin outside the testes and Geiser (1922) mentions "inconspicuous germ cells" migrating into the lobules from the soma of *Gambusia.* Regarding the source of the perch male germ cells, Turner (1919) states (p. 692): "A cord of germ cells outside the testes was found in a single specimen which was killed on May 5. Unfortunately, this was the only fish taken at this date and, though the cord has been sought in specimens taken at other dates, it has not been found." Foley (1927) believes that the male germ cells of *Umbra lima* come from stromal cells. Johnston (1951) on the largemouth bass, Wolf (1931) and Chavin & Gordon (1951) on the platyfish, and Essenberg (1923) on the swordtail depict a development of the testes essentially the same as that shown in the mullet. Essenberg finds, however, that some of the peripheral nests of germ cells (he refers to them as "primordial germ cells") degenerate and the fate of the others is uncertain. The duct epithelial cells divide and form spermatocytes, spermatids, etc., while the spermatocysts form within the ducts or tubules. Wolf (1931) disagrees with Essenberg's thesis; he reports that in the swordtail and platyfish, sperm are descendants of primordial germ cells and that duct epithelium does not form germ cells. Chavin & Gordon (1951) subscribe to this observation. Friess (1933) finds that duct epithelium of the swordtail is secretory; furthermore, she doubts if duct epithelium ever transforms into germinal cells. Goodrich, Dee, Flynn & Mercer (1934) state that the development of the testes in *Lebistes* is similiar to Wolf's description in the platyfish. Lavenda (1949), reporting on the protogynous hermaphroditism of the Atlantic sea bass. *Centropristes striatus,* reports that the testicular germ cells develop from the epithelial cells of the oviduct.

At one period during the early stages of the present study, before a complete series of specimens was examined, it appeared that the duct epithelium might be transforming into germ cells. Duct cells do increase in size and number

during growth and presence of germ cells along the base of the duct epithelium suggested such a transformation. Later, however, when a complete developmental series was examined, the independence of the two systems became obvious.

The Interstitium

The presence of endocrine interstitial cells has not been demonstrated in all fishes, either morphologically or physiologically. Courrier (1921) and Craig-Bennett (1930) state that the lymphoid tissue of the stickleback kidney and the endocrine interstitium of the testis are similiar in appearance. The lymphoid-appearing tissue of the mullet testis was examined carefully; no changes could be detected in the cytology of the elements during the maturation period. Possibly, as Craig-Bennett (1930) believes, osmic fixation is preferable to Bouin's fluid for demonstrating the granules, etc. James (1946b) describes clusters of cells in the interlobular tissue of largemouth bass testis, which she believes are glandular interstitial cells. Mathews (1938) reports "peculiar cells" in the testis of *Fundulus* which shrink after spawning. He interprets these cells as endocrine cells.

The interstitium of the mullet testis contained no cells which resemble the interstitial Leydig cells of common laboratory mammals.

Recently, Marshall & Lofts (1956) and Lofts & Marshall (1957) have reported that two distinct arrangements of the testicular endocrine cells occur in fishes. In the pike, char, *Labeo* and probably in some salmon, the glandular interstitium is in the lobules (tubules). The other type, reminiscent of the mammalian Leydig cells in which the endocrine interstitium rests between the tubules, is represented by the stickleback, sprat, *Tilapia* and some elasmobranchs. Marshall & Lofts (1956) report that the Leydig cells in both types are at first sudanophilic and then become cholesterol-positive after spawning.

Maturescence of the Ovary

The oocytes of *Mugil cephalus* are derived solely from germ cells; neither peritoneal (duct epithelium) nor stroma cells contribute to their numbers. Okelberg (1921), Hann (1917), Johnston (1951) and others have indicated that primordial germ cells are the sole progenitors of oocytes. Essenberg (1923) reports a unique condition to the swordtail. He believes that the primordial germ cells degenerate in potential female swordtails and that the functional oocytes are derived from peritoneal cells. Friess (1933) doubts that a somatic origin of oocytes exists in the swordtail and suggests that the marked degeneration reported by Essenberg is actually maturation. Wolf (1931) states that in *Platypoecilus maculatus,* oocytes have a duel origin; the greater number are derived from germ cells and the rest from somatic cells or perhaps from peritoneal cells. He reports some degeneration of germ cells, but not to the extent mentioned by Essenberg (1923) in the swordtail. Johnston (1951) has questioned the idea of a dual origin of oocyte and sperm. The point is well-taken. However, as Wolf (1931) intimates and as the studies on the mammalian ovary bear out (see references listed in the discussion of the origin of verebrate germ cells), evidence based on histological obesrvations has limitations, both technical and interpretative.

Goodrich, Dee, Flynn & Mercer (1934) point out the similarity of the development of the guppy and the platyfish and agree with Wolf (1931) that oocytes may arise from stroma cells, but that sperm originate only with germ cells. Dildine (1937), reporting on hermaphroditism in Lebistes, a phenomenon not mentioned by Goodrich, Dee, Flynn & Mercer (1934), did not find a transition of somatic cells to sperm or ova. Odum (1936) states that in *Opsanus tau,* oocytes develop from somatic cells as also does Guerbilsky (1939) in the mirror carp. On the other hand, Moore (1937), reporting on the rainbow trout, Robertson (1953) on the salmon, and Stromstein (1931) on the goldfish, declare that only germ cells give rise to oocytes.

Source of New Oocytes

Almost as controversial as the question of the origin of germ oocytes and sperm are the ideas on the source of new oocytes in the spent ovary. In the mullet, new oocytes are derived from the residual oogonia and germ cells in the periphery of the lamellae. Wheeler (1924), referring to the dab, Bullough (1939) to the European minnow and Craig-Bennett (1930) to the stickleback, report that some of the new oocytes are derived from the follicle cells of extruded oocytes. The concensus of those reporting on fishes appears to be that oogonia and young oocytes only form the new crop of oocytes and that the follicle cells degenerate.

Vitelline Body

Okelburg (1921) reports the presence of a vitelline body in the oocytes of the lamprey. James (1946a) mentions the presence of a yolk nucleus in the largemouth bass. Presumably, these structures are the same as the idiosome. The latter was visible in the oocytes until the time of the formation of the yolk particles.

Extrusion of Nucleoli

Essenberg (1923) and Stromstein (1931), reporting on the swordtail and goldfish, have described extensive extrusive nucleoli of oocytes. Essenberg states that the nucleoli of the helleri oocyte and indifferent germ cells pass into the cytoplasm and thence through the cell membrane into the follicle. As many as six nucleoli are seen in an oocyte. In the goldfish, Stromstein notes that nucleoli bud and divide into cytoplasm. He says (1931, p. 10): "This would seem to indicate that nucleolar extrusions are taking place at this time and that they are moving through the cytoplasm toward the periphery of the cells where a little later they form a definite zone." Comparable phenomena have not been observed in mullet oocytes. Oka (1931) believes that the nucleolus of the early normal *Oryzias* oocyte is replaced by scattered nucleoli, while in the "pseudocytes" (oocyte-like cells of males), the single nucleolus remains until a late stage. It has been impossible to confirm this nucleolar difference in the mullet.

Corpus Luteum

The literature contains reports on the appearance of corpus luteum-like structures in both live-bearing and egg-laying fishes. Samuel (1943) describes the development of structures in the elasmobranch, *Rhinobatus granulatus,* which are thought to be corpora lutea. Mathews (1938) believes that corpora lutea are present in the post-ovulatory follicle of *Fundulus heteroclitus.* Bretschneider & Duyvené de Wit (1947) also report corpora to be present in a number of teleosts and Selachii, describing the corpora as pre-ovulatory. Among the forms mentioned by these authors as possessing corpora is *Xiphophorus.* Friess (1933) describes "rest Korper" in the ovary and viscera of *X. helleri.* She believes that these bodies are formed from degenerate oocytes which have been invaded by special stromal cells, "Wanderzellen." She proposes that rest bodies may be involved in tumor formation. Chidester (1917) also found ovarian tissue scattered throughout the body of *Fundulus.*

Pickford & Atz (1957) have examined the evidence for the presence of corpora lutea in fishes; they state that no direct evidence has yet been submitted to show that the so-called corpora described by various investigators have a secretory function.

Fig. 23 from a partially spent *M. cephalus* ovary contains a great number of degenerating oocytes in varying degrees of atresia. Neither in this specimen nor in others was there any structure which bore any resemblance to the corpora lutea of mammals or to those reported in fishes.

Hermaphroditism

Numerous descriptions of hermaphrodite fish are encountered in the literature. Frequently, the instances of this phenomenon are reported as oddities or abberations. Thus the term "hermaphrodite" has taken on somewhat broad interpretations. In its strictest sense it describes the condition, as found in certain basses, wherein the gonad has a male and a female lobe or region which appear functional, possibly to the extent of being capable of self-fertilization. Hermaphroditism may also mean the quite common occurrence of varying numbers of oocyte-like elements in the embryo, the immature fish, or in the adult male. Finally, the term may describe sex reversal, protogyny or protandry.

Brock (1878) quotes Cavolini (1792) and others to the effect that certain of the sea basses are capable of self-fertilization. This claim has not been confirmed. However, the basses and related fishes have been shown to be unusual in their sex patterns. Van Oordt (1929) investigated *Serranus* and *Sargus,* and D'Ancona (1950) studied a number of serranids, sparids and related forms. Some of these fishes are stated to be functional hermaphrodites. D'Ancona (1950, P. 283) says: "J'ai pu constater chez *Serranus scriba* et *Hepatus hepatus,* la présence d'un hermaphrodisme functional constant . . ." Longley & Hildebrand (1940) report that certain basses from the Tortugas, Florida, region are functional hermaphrodites.

Cases of hermaphroditism in the embryonic or immature stages of fish, frequently manifested by the presence of oocyte-like cells in developing males, have been reported in the following forms: *Myxine glutinosa* (Cunningham, 1891-92 and 1886-87), Serranidae, Sparidae and the European eel (D'Ancona, 1945 and 1950), *Lebistes reticulatus* (Dildine[4], 1936), lamprey, *Entosphenus wilderi* (Okelberg, 1921), goldfish (Stromstein, 1931) and *Xiphophorus helleri* (Friess, 1933).

In the following species, hermaphroditism has been reported in the adult form (here again, the incidence of oocyte-like elements occurs in males): *Lepidosiren paradox,* (Agar, 1910), *Fundulus heteroclitus* (Chidester, 1917), *Bdellostoma* and *Myxine* (Cole, 1905, and Conel, 1917), trout (De Beer, 1924), *Phoxinus laevis* (Bullough, 1939), *Abramis brama* (Gryazeva, 1936), largemouth bass (James, 1946), *Carassius auratus* and *Sparus longispinis* (Kinoshita,

[4]Vaupel (1929), Goodrich, Dee, Flynn & Mercer (1934)and Berkowitz (1938) mention no hermaphroditism in the guppy.

1933 and 1936), *Fundulus majalis* (Newman, 1908), perch (Turner, 1928), *Oryzias latipes* (Oka, 1931), sea bream (Aoyama, 1955), *Serranus* and *Sargus* (van Oordt, 1929, and D'Ancona, 1945 and 1950). It should be noted that oocyte-like cells have been elicited experimentally by hormone and transplantation techniques (see Pickford & Atz, 1957).

Sex Anomalies in M. cephalus

Information on hermaphroditism in *Mugil cephalus* appears to be limited to a statement by Kesteven (1942) that "hermaphrodite roe are not very rare, one or two being found each season." Presumably these instances were based upon the gross examination of the gland. The present study demonstrates that anomalies in the gonads may not be detected by gross inspection. The presence of oocyte-like elements in immature glands or in testes are disclosed only in sectioned material. The hermaphrodite gland (Fig. 25) is a case in point. This ovary appeared to be quite normal; sections, however, revealed the presence of male elements.

Oocyte-like Cells.— In the mullet, oocyte-like cells appear in the undifferentiated gonad in specimens as small as 100 mm. Oocyte-like elements in the young fish never reach the size of those found in the mature males. They degenerate before the gland matures. As tube formation progresses in the males, oocyte-like cells continue to form. In the near-ripe, ripe, and spent testes, the oocyte-like cells are larger and more closely resemble true oocytes. The cytoplasm has the same basophilia and fine granular appearance as true oocytes. Whether this basophilia represents yolk precursor has not been determined. The other elements of the cell (nuclear membrane, nucleoli, lampbrush chromatin) are similar to young oocytes. Oka (1931) reports that the oocyte-like cells which he calls "pseudocytes" were restricted, in two *Oryzias* males, to a definite region of the testes and in a third specimen were distributed throughout the gland (as in the mullet). Oka (1931) finds that the "pseudocytes" develop in the same cyst with sperm. Agar (1910) also found this to be true of the lungfish. It appears to be true of the mullet.

D'Ancona (1945) states that oocyte-like cells of the eel and some other higher vertebrates are the result of an abbreviated oogenesis (oogénèse abrégée) in which such cells are formed directly from "protogonia" (primary germ cells) without the usual sequence of oogonia, etc. Observations on the mullet give some credence to this theory. In several specimens approximating 110 mm. in length, it appeared that the oocyte-like cells were most frequently seen in the peripheral nests. This might suggest that certain of the nest cells matured (?) precociously. As to why such cells should become feminized remains unanswered.

Okelberg (1921) reports in the lamprey and Grassi (1919) and D'Ancona (1950) in the eel that all gonads contain oocyte-like cells. Okelberg believes that the young lamprey is bisexual. Germ cells that show a tendency toward rapid division and formation of cysts are potentially male, whereas those that exhibit rapid growth are female. If both tendencies are equal, a temporary hermaphroditism occurs. When an imbalance in maleness or femaleness develops, the gonad becomes either male or female. When sex is finally established, the opposite sex elements gradually disappear. The undeveloped ova (oocytes) found in males represent the residual female elements of the bisexual stage. The condition in the eel is comparable (D'Ancona, 1950). Essentially this appears to be the pattern found in some other vertebrates, *e.g., Rana sylvatica, Sternotherus odoratus* and *Bufo lentiginosus*, as reported by Witschi (1921), Risley (1933) and King (1910). No adequate explanation for this seeming proclivity for femaleness in certain vertebrates has been offered.

Van Oordt (1929), Kinoshita (1936) and Lavenda (1949) have reported sex reversal (protogyny or protandry) as occurring in the sea basses and some related groups. Liu (1944) states that the symbranchid eel, *Monopterus javanensis,* is protogynous. All young fish are females and breed as such. Male germ cells develop in the ovary and eventually all females become functional males. Zwei (1950) describes three Mediterranean fish, *Maena samaris, M. chryselis* and *Paegellus erythrinus,* which are also protogynous. Regarding sex reversal in poeciliid fishes, Gordon (quoted by Pickford & Atz, 1957, p. 196) believes that Essenberg's (1926) claim of two cases of protogyny in *X. helleri* is open to some doubt, principally because no other examples of sex reversal have appeared in perhaps hundreds of masculinized swordtails that have been observed before and after 1926. Tavolga (1949) states that only two cases of sex reversal are recorded for the closely related platyfish.

Evidence from the present study does not indicate that the mullet is protogynous or protandrous. In the two examples of protogyny cited, the observers note that mature females are smaller than males. In the mullet, the opposite appears to be true. Several males from the May collection, measuring 175 mm., had residual sperm in the vas deferens. Females from the

February collection which measured 200 to 225 mm. were developing definite ovaries. The material used in this study suggests that normally females under 250 mm. in length do not spawn. A single ripening female in the October 28 collection measured only 150 mm. Mr. Malcolm Johnson, who made the collection, tells me that of the hundreds of mullets which he examined, this was the smallest ripening female seen.

Other investigators have suggested that *Mugil cephalus* males mature at a smaller size than females. Hotta (1955), reporting on *Mugil japonicus* (which, the author states, is believed to be identical with *Mugil cephalus*), finds that mature females have a body length greater than 45 cm. and that males are less than 40 cm. in length. Thomson (1951) examined 135 male and 92 female mullets more than 25 cm. in length, from Western Australian waters. No spent gonads were found in males smaller than 32 cm. or in females less than 35 cm. Broadhead (1953) reports that in the Gulf of Mexico, female mullets are larger than males. Of the mullets of the Salton Sea, California, Dill (1944) says that in a sample of large mullets, males averaged 16.5 in. (412 mm.) in length and females averaged 22.5 in. (562 mm.). Breder (1940), in describing the mating behavior of a group of mullets in a Florida creek, says that the males were about two-thirds the length of the females. Kesteven (1942) and others have suggested that the mullet male matures at an earlier stage than does the female. The histological evidence in the present study suggests that male mullets breed at the end of their second year. Females appear to be a year older. Bromhall (1954), speaking of the Hongkong mullets (*M. cephalus*), says that there is a primary and secondary spawning and he believes that the spawnings have a lunar periodicity. There seems to be some evidence to suggest that Florida mullets spawn more than once in a season. A number of females and males showed partially spawned gonads.

Conclusions

1. The germinal tissues of *Mugil cephalus* L. are derived from large germ cells which are found in the gonad anlage of the 20 mm. fry. The role of these cells in the formation of sperm and oocytes and their similarity to the primordial germ cells of other fishes suggests that they are primordial germ cells.
2. No evidence has been found to support the belief that peritoneal or other somatic cells transform into gametic elements.
3. Mullets exhibit a juvenile, sexually-indifferent stage.
4. Oocyte-like germ cells are found in varying amounts in the indifferent gonads and in the testes of mature and spent males.
5. The presence of oocyte-like cells in immature and mature male mullets suggests intersexuality rather than protogyny.
6. The gonad of a unique, hermaphrodite, mature mullet is described.
7. The evidence from this study suggests that male mullets mature sexually at a smaller size than do females. Males, it would appear, become mature at about two years of age; females first ripen during their third year.

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EXPLANATION OF THE PLATES

All figures are photomicrographs of preparations from Bouin-fixed material. Unless otherwise stated, the sections were stained with Galigher's Alum Hematoxylin and Triosin.

Plate I

Fig. 1 & 1a. Transverse section through the posterior abdominal region of a 23 mm. mullet fry. The arrow in Fig. 1 indicates the general position of the developing gonad between the abdominal cavity and the peritoneum. In Fig. 1a, the two anlagen are shown suspended from the black pigmented peritoneum and separated by the dorsal mesentery. The right anlage contains a small area of syncytial cord tissue. 24× and 530×.

Fig. 2. A 5-micra section through a genital strand of a 20 mm. fry. The large germ cell in the primordium measured approximately 12 micra on its long axis. 1300×.

Fig. 3. A 5-micra section (transverse) of one gonad from a 50 mm. fry. Note that the germ cells are limited to the lateral side and ventral end (left and bottom in the figure); also that the epithelial border is conspicuous on the lateral margin and lacking on the medial side. 1140×.

Fig. 4. A section through the dorsal mesentery from the posterior region of a 50 mm. fry. The peritoneum is at the top and the gut at the bottom of the figure. The two knob-like projections from the dorsal mesentery represent the genital strands in transverse section. 240×.

Plate II

Fig. 5. The arrangement of the germ cells around the lateral and ventral periphery (right and bottom) and the development of nests is indicated in this section from a 61 mm. specimen. The bottom of the figure shows top-shaped nests, an indication that some of the germ cells have migrated toward the center. Note the branched ducts which have penetrated to the peripheral nests. 495×.

Fig. 6. A transverse section of one gonad from a 75 mm. fish. The germ cells appear in nests along the lateral and ventral regions (left and bottom). The arrow indicates the hilus in which involution of the epithelial border is taking place. A 7-micra section. 450×.

Fig. 7. A transverse section from a 100 mm. mullet. The central or main duct has formed and appears as a narrow fissure in the figure.

Fig. 8. About one-half of a section of the gonad of a 150 mm. fish, collected in May, is shown. To the right and below center is a small, dark oocyte-like cell. 525×.

Plate III

Fig. 9. A low power view of a transverse section through the maturing testis of a 250 mm. mullet, collect in August. The tubules, containing the developing male cells, which radiate from the vas deferentia, are the light, columnar areas. Masson C. T. stain. 53×.

Fig. 10. Detail of several tubules from a testis similar to that in Fig. 9. A 7-micra section. Masson's C. T. stain. 525×.

Fig. 11. Maturation of the germ tissue in the peripheral region of a testis from a specimen collected in October. A 7-micra section. 525×.

Fig. 12. A general view of the peripheral region of a near-ripe testis collected in late October. The engorgement of the tubules with spermatids and sperm has reduced the stroma of the gland to no more than strands of tissue. A 5-micra section. 175×.

Fig. 13. A small area of a tubule of a near-ripe testis. A 5-micra section. 250×.

Plate IV

Fig. 14. An area from a spent testis of a 215 mm. fish. Note the two ova-like cells (right) and the germ cells in the circled area. A 7-micra section. 250×.

Fig. 15. A low power view of an area from a spent testis collected in February (275 mm. standard length). The crenated edge of the gland and the collapsed tubules are characteristic of the spent testis. A 7-micra section. 53×.

Fig. 16. An ova-like cell surrounded by sperm. The region shown is the vas deferens. From the same fish as Fig. 15. A 7-micra section. 495×.

PLATE V

FIG. 17. A transverse section of a developing ovary from a 200 mm. specimen collected in February. The arrangement of the germ cells in nests is characteristic of the presumptive ovary. A 7-micra section. 185×.

FIG. 18. A low power view of a more mature ovary. This specimen measured 200 mm. and was collected in February. Note that the duct that separates the body of the gland from the mesovarium, which forms the vas deferens in the male, is still present. A 7-micra section. 53×.

FIG. 19. A small area from the same ovary as Fig. 18. There are nests of maturing gonia cells and oocytes in various stages of development. A 5-micra section. 250×.

PLATE VI

FIG. 20. A low power photograph of a section through the peripheral region of an ovary collected in February. The general pattern of the lamellae is shown. The tunica in this region is still made up of two layers of collagenous connective tissue. (An area from a region near the mesorchium would have shown smooth muscle in the tunica). Note the pigmented serosa. A 7-micra section. 53×.

FIG. 21. Sections through several near-ripe oocytes from the ovary of the largest female fish examined, *i.e.*, 425 mm. This fish was collected on February 15. The dark, irregularly-shaped structures near the top of the figure are young oocytes which have been distorted by the pressure of the larger oocytes. A 7-micra section. 250×.

FIG. 22. A higher magnification view of the cortical regions of three ova from the same specimen as the previous figure. Only two structures appear to be outside the cytoplasm of the egg, a zona radiata and the follicle cells. The round clear areas in the cytoplasm are presumably the result of oil dissolution. Masson's C. T. stain; 6-micra section. 525×.

PLATE VII

FIG. 23. A low power view of a section through the ovary of a spent fish. A 7-micra section. 53×.

FIG. 24. A small area from a spent ovary of a fish of 275 mm. collected in February. Between the dark oocytes are two or three nests of germ cells, probably oogonia. A 7-micra section. 525×.

PLATE VIII

FIG. 25. A low power view of a transverse section through one gonad of an hermaphrodite specimen. It was collected on October 8. A 7-micra section. 53×.

FIG. 26. From the above specimen, showing spermatids and sperm in the testicular part of the gland. Note the dark ova or oocyte-like cells as well as normal-appearing sperm "parachutes". A 7-micra section. 600×.